CYTOLOGICAL AND CHROMATOGRAPHIC EVIDENCE FOR INTERSPECIFIC HYBRIDIZATION IN PETALOSTEMON[1]

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ABSTRACT

Chromatographic profiles of flavonoid extracts of *P. purpureum* and *P. gattingeri* are quite distinct, *P. purpureum* showing 12 species-specific compounds, and *P. gattingeri* showing 6 specific compounds. Chromatographic profiles of all putative hybrids except one exhibit compounds specific to each parental species. Three types of chromosomal abnormalities exist in putative hybrids which are not present in either parental species, but completely normal meiosis is reported in several hybrids.

In a revision of the genus *Petalostemon* Michx., Wemple (1965) reported that interspecific hybridization is possible within taxonomic sections of this genus but not between species of different sections. He created fertile, artficial hybrids between *Petalostemon gattingeri* (Heller) Heller and *P. purpureum* (Vent.) Rydb., members of sect. *Purpurei*. In nature, these species are usually ecologically isolated. *Petalostemon gattingeri* is endemic to limestone glades in Tennessee and Alabama, and *P. purpureum* is a prairie species.

In the central basin of middle Tennessee, *Petalostemon gattingeri* is widespread on limestone glades, while only a few individuals of *P. purpureum* have been found in the area. Naturally occurring populations of putative hybrids of these two species were observed by Breeden (1968) in Cedars of Lebanon State Park in Wilson County, Tennessee. In these populations, *P. gattingeri* was found along a roadside and on patches of open limestone. *Petalostemon purpureum* was found in adjacent areas which Breeden considered to be prairie relicts. He measured fourteen morphological characters of the two parental species and of putative hybrids and found that the hybrids were generally intermediate between the two parental species or within the range of variation of one of them. The present study was undertaken to supplement morphological data with chromotographic and cytological data.

MATERIALS AND METHODS

The following populations of the parental species and hybrids were studied:

P. gattingeri

Davidson County, Tennessee: Mount View. *Walker 195*

Davidson County, Tennessee: Couchville Pike. *Walker 196*.

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P. purpureum

Sevier County, Arkansas: Ben Lomond. *Demaree 53998.*

P. purpureum × *gattingeri*

Wilson County, Tennessee: Cedar Forest Road. *Walker 176.*

Wilson County, Tennessee: Hurricane Creek Road. *Walker 190.*

Voucher specimens from these populations are deposited in the Vanderbilt University Herbarium (VDB).

Chromatography. Chromatographic patterns of flavonoid compounds in the two parental species were established from plants collected from pure populations in which there was no evidence of hybridization. Ten plants of each parental species and ten plants from each of the two putative hybrid populations were analyzed. Flavonoid compounds were extracted from stems and leaves in 99% methanol : 1% normal HCl in a ratio of one gram dry weight of plant material : ten ml. extracting solvent. Twenty drops of extract were spotted on a sheet of Whatman #3 MM chromatographic paper which was developed in the first direction in a solution of n-butanol: acetic acid: water (6:1:2) for seven hours and in the second dimension in 15% acetic acid. The chromatograms were observed under ultraviolet light both before and after the application of ammonia fumes. The chromatograms were then dipped into a solution of one part 1% potassium ferricyanide and one part 2% ferric chloride, in dilute HCl, and finally in water. The spots were identified by means of the Rf values in two dimensions and color reactions with the treatments described above.

Cytology. Inflorescences were fixed in absolute ethanol: glacial acetic acid: chloroform (9:3:1). After fixation, the material was transferred to 70% ethanol and stored at —15° C. Pollen mother cells were stained with iron-propionic carmine. The pachytene stage, as well as later stages of meiosis, was examined in putative hybrids and in the parental species.

RESULTS

Chromatography. A list of Rf values and color reactions of compounds found in *Petalostemon gattingeri* and *P. purpureum* is given in Table 1. Twelve compounds are specific to *P. purpureum* and six compounds specific to *P. gattingeri.* The chromatographic profile of *P. purpureum* is shown in Figure 1A and that of *P. gattingeri* in Figure 1B. With the exception of one plant, every putative hybrid was found to have some compounds specific to each of the two species. However, no single plant exhibited all of the compounds specific to each species. Table 2 shows the number of plants in which each species-specific compound occurred in each population.

Cytology. Meiosis was found to be completely normal in pollen mother cells of the parental species. The pachytene stage of meiosis is particularly useful for karotype analysis in this genus. Figures 2 and 3 show pachytene cells of *Petalostemon gattingeri* and *P. purpureum,* respectively. The nucleolus and nucleolus organizer are prominent, as well as chromatic and achromatic regions of the seven pairs of chromosomes. No significant differences were found in the karyotypes of the two species (Walker, unpublished).

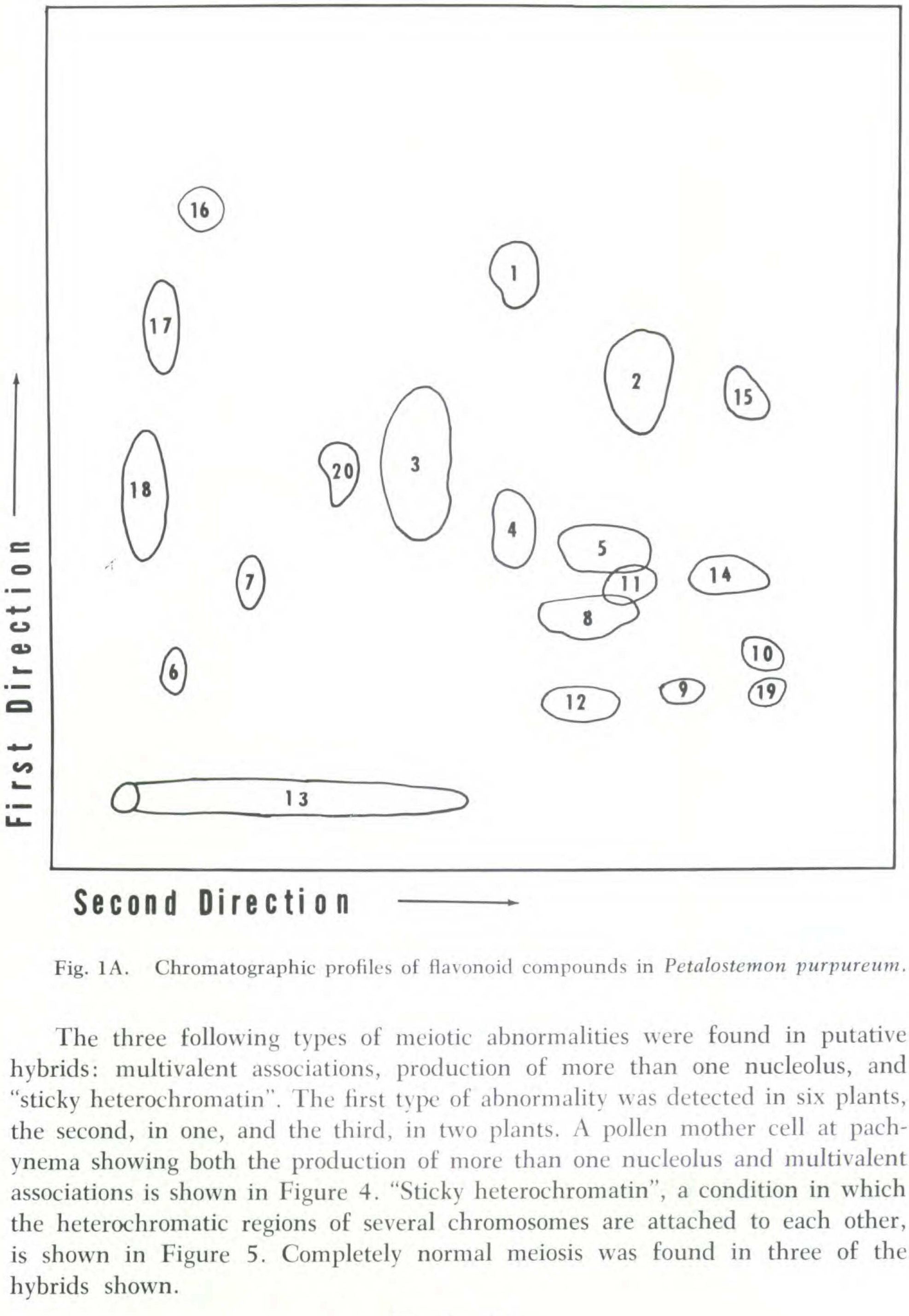

Fig. 1A. Chromatographic profiles of flavonoid compounds in *Petalostemon purpureum*.

The three following types of meiotic abnormalities were found in putative hybrids: multivalent associations, production of more than one nucleolus, and "sticky heterochromatin". The first type of abnormality was detected in six plants, the second, in one, and the third, in two plants. A pollen mother cell at pachynema showing both the production of more than one nucleolus and multivalent associations is shown in Figure 4. "Sticky heterochromatin", a condition in which the heterochromatic regions of several chromosomes are attached to each other, is shown in Figure 5. Completely normal meiosis was found in three of the hybrids shown.

Discussion

Wemple (personal communication) found meiosis to be regular in artificially produced F_1 hybrids between *Petalostemon gattingeri* and *P. purpureum*, indicat-

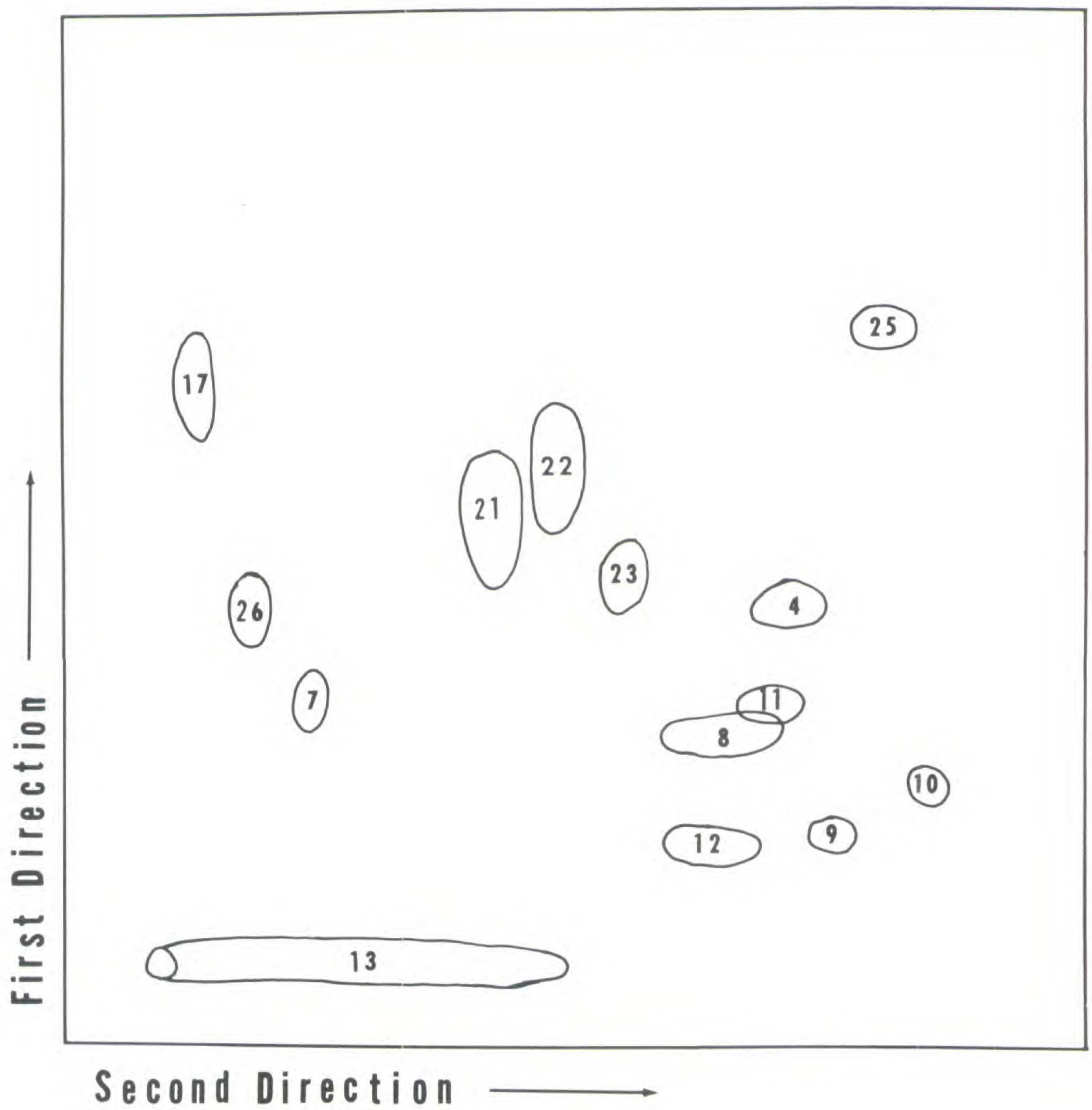

Fig. 1B. Chromatographic profiles of flavonoid compounds in *Petalostemon gattingeri*.

ing that there are no major chromosomal differences between these two species. Karyotype analysis (Walker, unpublished) has also shown no differences in chromosome structure of the parents. The occurrence of multivalent formation in hybrid derivatives is therefore indicative of genic disharmony.

The presence of two nucleoli in hybrid derivatives is also indicative of genic disharmony. Navaschin (1934) showed that in interspecific hybrids of *Crepis*, there is competition among nucleolar organizers. Nucleolar organizers of different species differ in their competitive abilities, and in interspecific hybrids the stronger nucleolar organizer suppresses the weaker one. In hybrids between species which have organizers of about the same strength, both organizers function, and two nucleoli are produced. It seems likely that in *Petalostemon gattingeri* and *P. purpureum*, one of the two nucleolar organizers is normally suppressed but that in

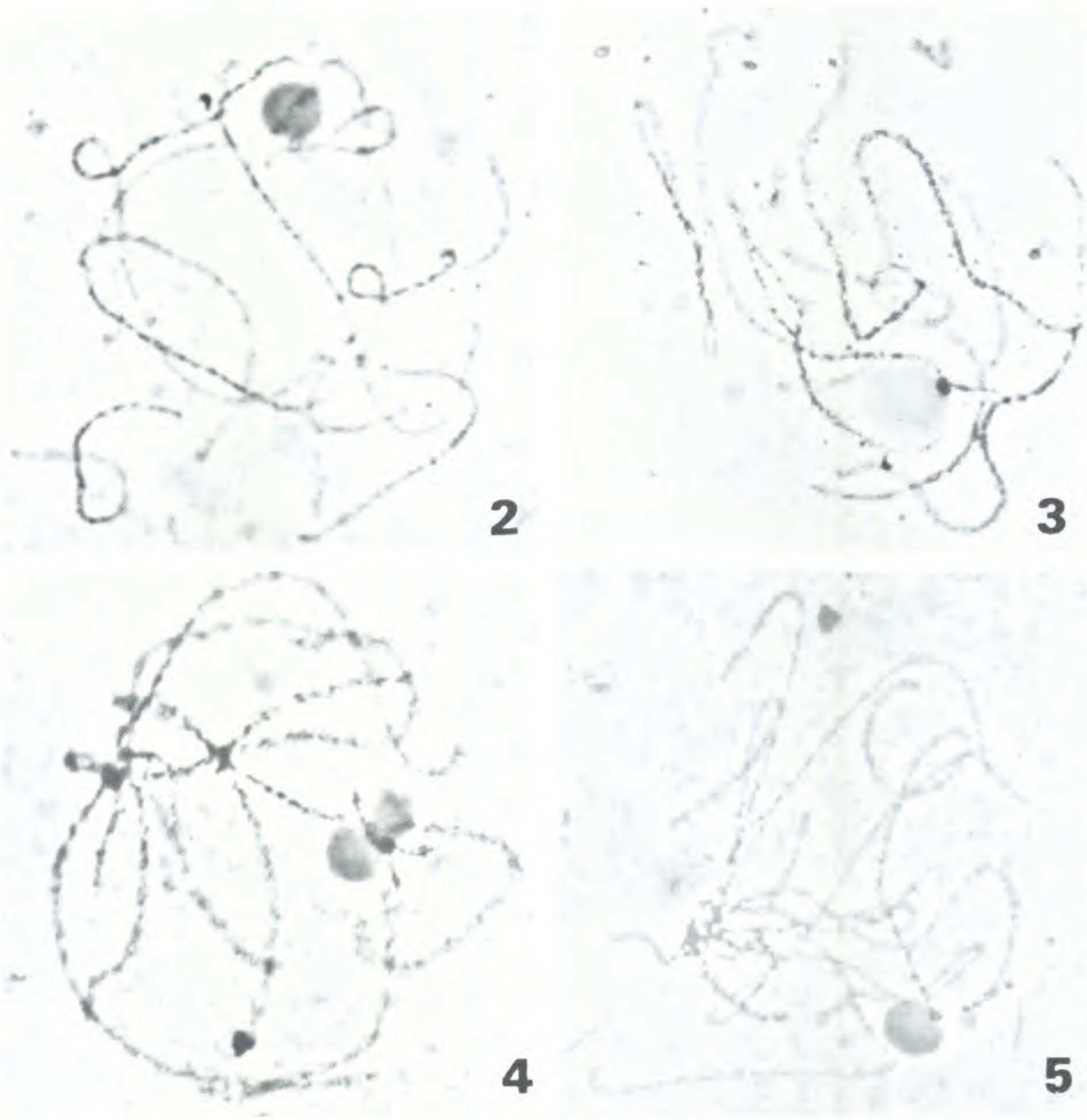

Fig. 2-5. Pachytene stage of meiosis in pollen mother cells. Fig. 2. *Petalostemon gattingeri,* × 868. Fig. 3. *P. purpureum,* × 1073. Fig. 4. Hybrid, × 1195. Fig. 5. Hybrid, × 1200.

some hybrid derivatives this suppression does not occur because of the particular gene combination present.

The third type of abnormality, sticky heterochromatin, may also be the result of genic imbalance. Nielsen (1961) has found this condition in meiotic prophase derivatives of *Agroelymus turneri,* a putative hybrid of *Agropyron dasystachyum* and *Elymus innovatus.* This condition, described by Nielsen as "accumulation of pycnotic materials," produced lethality in the microsporocytes. He concluded that this abnormality may be the result of an unbalanced enzyme and nucleoprotein condition.

Since no structural differences in the chromosomes of *Petalostemon gattingeri* and *P. purpureum* were revealed by karyotype analysis, and since meiosis in the F_1 hybrids is regular, it is concluded that abnormalities in hybrid derivatives of

TABLE 1
COMPOUNDS FOUND IN PARENTAL SPECIES

Spot #	Rf, 1	Rf, 2	UV	UV + NH_3	$FeCl_6$ + $K_3Fe(CN)_6$	P*	G*
1	0.74	0.55	white	—	—	+	—
2	0.59	0.73	light blue	—	—	+	—
3	0.48	0.41	purple	yellow	+	+	—
4	0.39	0.55	purple	yellow	+	+	—
5	0.36	0.69	purple	—	—	+	—
6	0.19	0.07	white	—	—	+	—
7	0.31	0.18	yellow	—	—	+	+
8	0.26	0.66	purple	yellow	—	+	+
9	0.16	0.79	white	—	—	+	+
10	0.21	0.90	white	—	—	+	+
11	0.32	0.72	white	—	+	+	+
12	0.14	0.65	—	—	+	+	+
13	0.02	0.27	pink	—	+	+	+
14	0.33	0.85	—	—	+	+	—
15	0.57	0.88	pink	—	+	+	—
16	0.83	0.10	bright blue	—	—	+	—
17	0.66	0.05	pink	yellow	—	+	+
18	0.45	0.02	light blue	yellow	—	+	—
19	0.11	0.91	white	—	+	+	—
20	0.46	0.30	white	—	—	+	—
21	0.53	0.39	purple	—	+	—	+
22	0.57	0.47	light blue	—	—	—	+
23	0.46	0.55	purple	—	+	—	+
24	0.42	0.74	light blue	—	—	—	+
25	0.74	0.85	—	—	+	—	+
26	0.41	0.11	yellow to pink	—	—	—	+

* P, compound found in *Petalostemon purpureum;* G, compound found in *P. gattingeri.*

TABLE 2
OCCURRENCE OF SPECIES-SPECIFIC COMPOUNDS IN PUTATIVE HYBRID POPULATIONS

	Compound Specific to *P. purpureum*	Specific to *P. gattingeri*	No. of plants in population #1 with compound (total: 10 plants)	No. of plants in population #2 with compound (total: 10 plants)
1.	+	—	6	4
2.	+	—	4	1
3.	+	—	8	8
4.	+	—	5	8
5.	+	—	3	7
6.	+	—	8	10
14.	+	—	3	0
15.	+	—	1	2
16.	+	—	6	9
18.	+	—	6	10
19.	+	—	7	6
20.	+	—	2	4
21.	—	+	5	6
22.	—	+	2	4
23.	—	+	6	6
24.	—	+	5	4
25.	—	+	3	1
26.	—	+	4	2

these two species are the result of genic disharmony and not the result of structural chromosomal differences.

The chromatographic data provide evidence for natural hybridization between these two species and support Wemple's conclusion that barriers to hybridization are ecological and not genetic.

Anderson (1949) has pointed out that the evolutionary role played by introgressive hybridization is the enrichment of variation in the participating species. In considering the significance of hybridization in the two populations studied here, one must take into account that *Petalostemon gattingeri* is much more abundant in these areas than *P. purpureum* since the limestone glade habitat of *P. gattingeri* is more prevalent in the area than is the typical prairie habitat of *P. purpureum*. Therefore it appears that the consequences of introgression will be an increase in the variability of the *P. gattingeri* and a gradual disappearance of *P. purpureum* individuals from the population.

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